REMARKS/ARGUMENTS

Claims 28-29 and 31-54 are pending.

Claim 30 has been cancelled.

Claims 45 and 47-54 have been withdrawn.

Applicants wish thank the Examiner for indicating that claims 43-44 are allowable.

Claims 28, 31-42, and 46 are rejected under 35 U.S.C. 103(a) over Oh et al., US 5,851,778, Lee et al., (*J. Agr. Food. Chem.*, 1999, vol 47, p. 2766-2770), and Groopman et al., PNAS, 81:7728-31 (1984). The rejection is traversed because the combination of the references does not describe:

1) a trifunctional reagent;

2) a regeneration of a solid support;

3) measuring the intensity of a signal emitted by a luminescent group L on a solid support, which is proportional to the amount of an analyte to be detected; and

4) one would not have been motivated to used the Lee et al. solution-based fluorescent excitation transfer assay in the Oh et al. solid support assay because the Oh et al. and Lee et al assays are based on different principles. Also, combining the assays of Oh et al. and Groopman et al. is improper because Oh et al.'s method requires the reaching of an equilibrium of the reagents, while Groopman et al. describe a thorough washing of a column.

Subject matter and advantages of the present invention

The invention defined by claim 28 relates to a method for detection of an analyte a in a fluid sample, comprising:

1) saturating a solid support comprising, on at least part of its surface, at least one **trifunctional** reagent (tripod Y) comprising the following three functional poles:

i) a **luminescent group (L)**,

ii) a **molecule (B) chosen from the analyte a, an analog of the analyte a or a fragment of the analyte a**; and

iii) **a function** that provides **attachment** of the trifunctional reagent to the surface of the solid support,

with a receptor for the analyte a, the receptor being **labeled with a compound (Q) (receptor-Q) that quenches the luminescence of the group L**, so as to form a complex C between the molecule (B) and the receptor-Q;

2) bringing the solid support obtained in step 1) into contact with a fluid sample that may comprise the analyte a to be detected;

3) **measuring the intensity of the signal emitted by the group L**, which is proportional to the amount of analyte a present in the fluid sample; and

4) regenerating the solid support by bringing the solid support into contact with the receptor-Q.

The claimed method is a method for continuous, **heterogeneous-phase detection** of analytes in a fluid sample that makes possible to avoid any incubation, prior to the detection step per se, of the analyte with a labeled antibody or a labeled analyte. Claim 28 specifically mentions that steps 3) and 4) are carried out continuously.

According to the claimed method:

- step 1) allows complexation of the molecule (B) with the receptor-Q. At the end of this first step, the luminescence of L is decreased or suppressed.

- In step 2), bringing the sample into contact with the surface of the solid support will induce, when said sample contains the analyte a, **competition** between the analyte a and the molecule (B) for the formation of the complex with the receptor-Q. The binding of the analyte a to the receptor-Q will result in the **receptor-Q being eliminated from the surface**

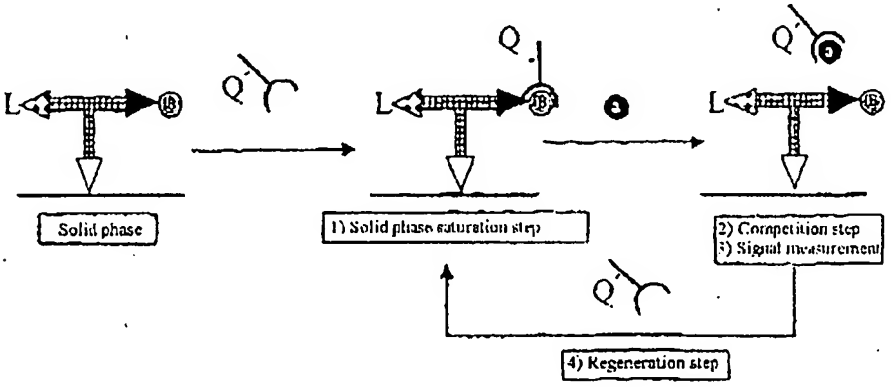
of the solid support and in the luminescence emitted by the compound (L) present on the tripod Y being restored.

- The intensity of the signal measured in step 3) is then proportional to the amount of analyte a present in the sample to be analyzed.

- The regeneration step 4) will again bring about complexation of the receptor-Q on the tripod Y and thus suppression of the luminescence of L, so as to allow further detection of the analyte a in a new sample. No intermediates washing steps are necessary between steps 3) and 4).

The principle of the method for detection of an analyte a in accordance with the invention is represented diagrammatically in scheme A below:

SCHEME A



The method of detection in accordance with the invention has a large number of advantages:

1) due in particular to the specific structural conformation of the tripod Y used during the method, the step consisting of **regeneration of the solid phase can be carried out very readily**, without any alteration of its properties. In fact, in all the flow assays described according to the invention, the signal is measured after formation of a complex bound to the solid phase. Consequently, and even though some of the assaying methods described by the

prior state of the art allow several successive assays, they are however limited in number and require a solid support regeneration step that is often long and restricting, which results in the dissociation of the complex formed. In addition, the drastic conditions for carrying out these regeneration steps (passing over acid or basic solutions) mean that the detection method must be stopped, thus prohibiting any application of these methods to continuous assays of a given analyte.

In the claimed method, the presence of the analyte a in the sample brings about dissociation of the receptor-Q and of the molecule (B); **the regeneration step therefore consists quite simply in reforming this complex by adding receptor-Q.** This regeneration step does not, therefore, involve the use of acid or basic solutions that may impair the properties of the molecules of the solid phase or an exchange reaction between two molecules at the receptor binding site, the kinetics of which are longer than the reaction to form a complex

2) the signal is measured in the region on which the tripod Y was immobilized, which makes it possible to obtain a localized signal, unlike the assays developed according to the prior art, in which the signal, bound to molecules in solution, is measured at the outlet of a capillary,

3) since the signal is localized, several molecules may be detected simultaneously on the same solid support by attaching, to distinct and known zones thereof, several types of tripods Y that differ from one another through the nature of the molecule (B) that they comprise,

4) the signal measured corresponds to all the molecules of analyte a that have been in contact with the solid support between two regenerations. This particularity of the method in accordance with the invention allows permanent monitoring to be obtained while at the same time taking measurements that are spaced out over time,

5) this assay format is applicable to all molecules since it does not require the simultaneous binding of two receptors to the analyte, as is sometimes necessary according to the assay methods previously known, and which require the analyte to be of sufficient size,

6) the presence of analyte a in the sample results in the appearance of a signal, unlike most of the competition assays known from the prior art, the appearance of a signal allowing easier detection,

7) finally, since the detection system uses the energy transfer phenomenon, it also makes it possible to detect and to quantify the presence of an analyte a by means of the variation in luminescence of the compound Q if it is fluorescent or the variation in the apparent time for decrease in luminescence of the compound (L).

Disclosure of the cited references

Oh et al. teach a trifunctional conjugate comprising three chemical moieties attached through a spacer moiety. The method of use of those conjugates relate on the principle of steric hindrance.

In the Oh et al. assay, a sample is contacted with the conjugate, a limited quantity of analyte binding partner and an excess of small molecule binding partner. A presence of the analyte is determined by detecting the amount of an analyte binding partner diverted away from the analyte attached to the spacer of the conjugate (abstract).

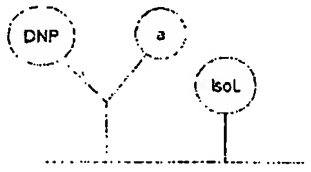
Most embodiments are concerned with conjugates in solution which are not relevant to the claimed method.

A variant including a solid support is disclosed in figure 11, col. 18, l. 20-50 and example 8.

Two of the tridentate members are small molecules ligands, like theophylline and DNP. The third tridentate member is a solid support. According to that variant, it should be

noted that the Luminescent group (L) is directly linked to the solid support and not to the trifunctional reagent.

The variant of figure 11 works according to the following scheme:



Scheme A

The solid support is functionalized by a tridentate conjugate comprising an analyte ⓐ (theophylline), a small molecule ligand DNP and a function that links the trifunctional reagent to the solid support.

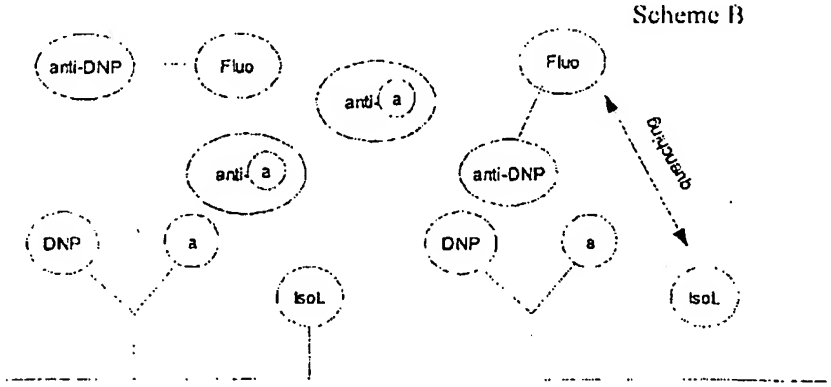
A luminescent molecule (IsoL) or isoluminol is directly linked to the solid support (co. 18, l. 39-45).

Thus, it cannot be said that the trifunctional reagent comprises a luminescent group.

A proximity label, like a luminescent molecule fluorescein, is attached to the DNP antibody.

The fluorescein-DNP antibody and a theophylline antibody are incubated with the functionalized solid support.

Scheme B



The anti-ⓐ antibody and the anti-DNP antibody are in competition to be attached to their respective ligands. At equilibrium, a certain quantity of each antibody is attached to its respective ligand.

Where the anti-DNP-Fluorescein ligand is attached to DNP, the fluorescein quenches the isoluminol luminescent molecule. Steric hindrance prevents the attachment of both antibodies to one sole trifunctional conjugate.

Then the analyte ⓐ to be detected is introduced.

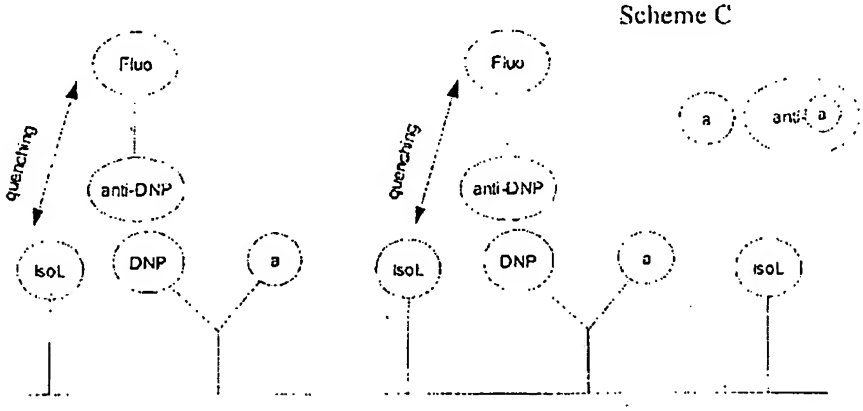
There is a competition between the analyte ⓐ in solution and the analyte of the tridentate.

A new equilibrium must be reached:

Anti-ⓐ antibodies liberate ⓐ ligands comprised in the tridentate, and link to the ⓐ in solution. The liberated tridentates are free to conjugate to anti-DNP antibodies.

More anti-DNP antibodies are linked to their DNP ligands. As DNP antibodies bear a luminescent molecule, this results in more quenching of the isoluminol. So that the luminescence is <u>inversely proportional</u> to the concentration of the analyte ⓐ (theophyllin).

Scheme C



It can be noted that numerous steps are necessary to perform such a measurement: optimum reagent concentrations have to be determined (anti-a antibody and anti-DNP antibody - see col. 38, l. 22 - col. 39, l. 5).

It is specially indicated that in the case wherein a solid support is used, incubation times have to be extended (col. 39, l. 35-52).

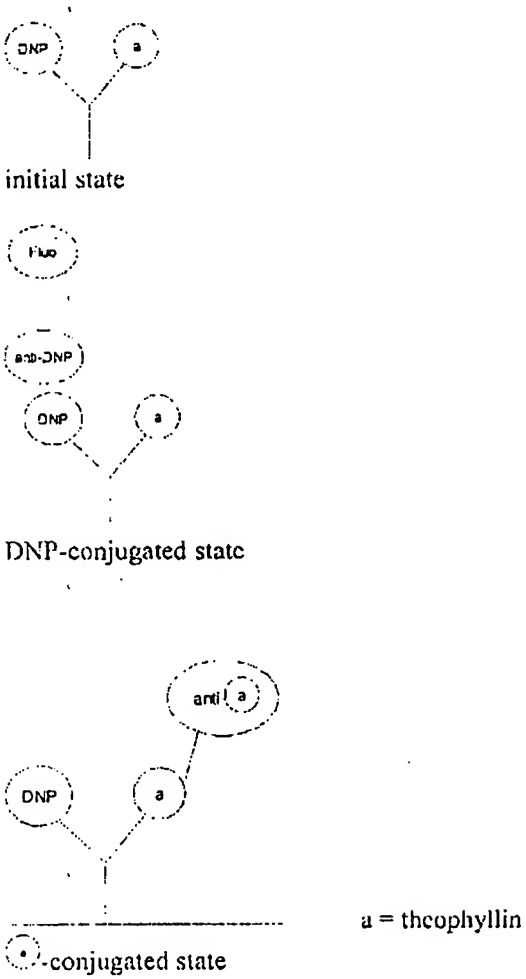
Finally, no indication is given of a possibility of regenerating the solid support.

When the method of Oh et al. is compared to claim 1, the following comment can be made:

If the luminescent group (L) is considered analogous to IsoL, then it cannot be said that the trifunctional reagent comprises a luminescent group. IsoL is directly linked to the solid support and is not part of the trifunctional reagent.

The fluo-anti DNP antibody could be considered as forming with its DNP ligand this luminescent part of the trifunctional reagent.

But trifunctional conjugates in Oh et al. can be in three different states:



In the initial state, the trifunctional conjugate of Oh et al. does not respond to the characteristics of the tripod Y of claim 28 (L is missing).

In the DNP-conjugate state, a saturation with the receptor for the analyte ② is not possible on account of steric hindrance: ② remains free of its antibody.

In the ②-conjugated state, the luminescent groups is absent.

So that whatever their state, none of the trifunctional conjugates of Oh et al. correspond to the saturated trifunctional reagent of claim 28.

Oh et al. rely on steric hindrance to reach an equilibrium between conjugates. The result, as already detailed above, is that the signal is inversely proportional to the quantity of the analyte.

This is another difference with the method of the invention wherein the intensity of the signal emitted by L is proportional to the amount of analyte.

Finally, bringing the solid support into contact with the ⓐ-antibody would not regenerate the solid support:

For each experiment an optimum reagent concentration for energy transfer must be reached (see col. 38, 1. 22 and following). Adding the anti ⓐ antibody would not permit a restoration of that equilibrium.

It should be stressed that the necessity of determining these equilibrium conditions makes the method very long and complicated.

Although no indication is given, it can only be imagined that regeneration can be obtained by complete washing of the solid support.

Thus, a continuity between steps 3) and 4) is not possible with the method of Oh et al.

To summarize, the most important differences between Oh et al. and the invention are listed hereunder:

- The trifunctional conjugates grafted supports of Oh et al. are different from that of claim 28.

- The intensity of the signal measured in Oh et al.'s solid support variant is inversely proportional to the presence of the analyte to be detected.

- The step of a regeneration of the solid support of Oh et al. cannot be performed continuously with steps 3) by bringing the solid support into a contact with the anti ⓐ antibody.

- And Oh et al. do not disclose that an analyte specific receptor is labeled with a compound that quenches luminescence.

The Examiner is of the opinion that the last characteristic is supposed by Lee et al. However, combining the teachings of Oh et al. and Lee et al. is improper because:

The assay of Oh et al. is based on a solid support grafted by a trifunctional conjugate.

Further, the disclosure of Lee et al. relates to a homogeneous method of immunoassay, i.e., to a method carried out in a liquid medium. The claimed method is a method for the heterogenous-phase detection of analytes in a fluid sample carried out on a solid support.

Oh et al. rely on a signal which is inversely proportional to the concentration of the analyte and Lee et al. relies on a signal which is proportional to that concentration.

Oh et al. rely on an equilibrium between two ligand-antibody systems, whereas Lee et al. relies on a direct relationship between ligand and receptor.

Even if Lee et al. disclos a method according to which the fluorescence of a receptor is quenched by a reporter, it does not cure the deficiencies of Oh et al., because Oh et al. describe trifunctional conjugates grafted supports which are different from that claimed.

Lee et al. do not describe or suggest a regeneration of a solid support.

Groopman et al. describe a method for a regeneration of monoclonal antibody affinity columns. An antibody is covalently bound to a sepharose column and used in an immunosorbent assay.

The regeneration of the column is detailed on p. 7730, col. 2, 1st paragraph: "Sequential washes with phosphate buffer (pH 3.0), diethanolamine buffer (pH 9.9), and phosphate buffer (pH 2.0) removed <1% of the applied [3H]AFB_1. However, quantitative elution of the bound [3H]AFB_1 was achieved by using 50% dimethyl sulfoxied (Me_2SO) in

phosphate buffer (pH 7.4) (50% Me_2SO buffer). The antibody column was regenerated by washing the column with P_i/NaCl (pH 7.4)."

Such a treatment in rather severe acidic conditions is what the skilled professional would actually use to regenerate the Oh et al. solid support. But such a treatment does no correspond to the characteristic of claim 28 "bringing the solid support into contact with the receptor-Q,", and applying this treatment between step 3 and step 4 would be in contradiction with the characteristic "when step 3) and step 4) are carried out continuously."

As has been explained previously, Oh et al.'s method requires the reaching of an equilibrium of the reagents (antibodies to the two ligands). It cannot be combined with a thorough washing of the column in a manner which could be qualified as "continuous".

The conclusion is that the method of claim 28 is inventive with regards to the disclosure of Oh et al., Lee et al., and Groopman et al. because:

Combination of Oh et al. and Lee et al. is improper the assays are fundamental different, and, even if combined, certainly the assays do not result in the claimed method.

A combination of Oh et al. and Groopman et al. certainly does not result in the claimed method.

Thus, Oh et al., Lee et al., and Groopman et al. do not make the claimed method obvious. Applicants request that the rejection be withdrawn.

Claims 29 is rejected under 35 U.S.C. 103(a) over Oh et al., Lee et al., Groopman et al., and Plowman et al., Anal. Chem., 71:4344-52 (1999). The rejection is traversed because the combination of the references does not describe:

(1) a trifunctional reagent;

(2) a regeneration of a solid support;

(3) measuring the intensity of a signal emitted by a luminescent group L on a solid support, which is proportional to the amount of an analyte to be detected; and

(4) one would not have been motivated to used (a) the Lee et al. solution-based fluorescent excitation transfer assay and (b) an integrated optical waveguide used to evanescently excite fluorescence from a multianalyte sensor surface in a sandwich assay; multiple channels; pre-mixed analytes and labeled tracer antibodies of Plowman et al.,. in the Oh et al. solid support assay because the Oh et al, Plowman et al., and Lee et al. assays are based on different principles.

The disclosurea of Oh et al., Lee et al., and Groopman et al. are described above.

Plowman et al. do not cure the deficiency. Plowman et al. describe a sandwich immunoassay for detecting multiple analytes (fig. 1) in which a capture antibody is attached to a solid support and a pre-mixed analyte with a tracer antibody labeled with a dye is passed over the capture antibody (fig. 1; Material and Methods, pages 4346-47).

Plowman et al. do not describe tripoids as claimed and a regeneration of a solid support ("regeneration of the surface was not considered," page 4347, right col., line 10). Thus, substituting the Plowman et al. assay into that of Lee et al. and Oh et al. assay still does not produce the claimed method.

In addition, Plowman et al. describe an assay based on a completely different principle (e.g., integrated optical waveguide used to evanescently excite fluorescence from a multianalyte sensor surface in a sandwich assay; multiple channels; pre-mixed analytes and labeled tracer antibodies) and uses a different set up and reagents compared to Oh et al. and Lee et al. so that detecting multiple analytes in the Oh et al. and Lee et al. assay by using the principle of Plowman et al. is not possible.

Thus, Oh et al., Lee et al., Groopman et al., and Plowman et al. do not make the claimed method obvious. Applicants request that the rejection be withdrawn.

A Notice of Allowance for all pending claims is requested.

Respectfully submitted,

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